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Original Article

MOLECULAR CHARACTERIZATION OF SUGARCANE GENOTYPES USING SSR MARKERS

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ABSTRACT

One of the most important industrial crops in the world is sugarcane. Genotype identification of sugarcane at a molecular level to eliminate qualm that are often associated with phenotypic descriptions. In this study, three varieties of Sirugamani genotypes (TNAU Sugarcane Si. 6, TNAU Sugarcane Si. 7, TNAU Sugarcane Si. 8) and Coimbatore genotypes (Co 86032, Co 99004, Co 99006, Co 94008, Co 94012, Co 2001-13 and Co 2001-15) were characterized with set of five SSR primers with high polymorphism information content. Among the five SSR primers, one primer (UGSM36) was found to be dimorphic and remaining four were polymorphic. The Polymorphism Information Content (PIC) value ranged from 0.36 to 0.79. The Cluster analysis clustered genotypes into three distinctive clusters with similarity coefficient ranged from 50% to 83%. The primer UGSM49 clearly divided the genotypes location wise. Sirugamani genotypes could be separated from Coimbatore cultivars by using this primer and it was unique primer for varietal identification. It was concluded that, SSR markers can help to identification and characterization of sugarcane genotypes.

KEYWORDS: Sugarcane, Sugarcane Genotypes & SSR Markers

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INTRODUCTION

Sugarcane is a large perennial grass cultivated in tropical and intertropical regions. It belongs to the genus *Saccharum L.* of the family Poaceae, which is placed in the tribe *Andropogoneae* (Jannoo *et al.*, 1999) together with the genera *Zea* and *Sorghum* (Lu *et al.*, 1994). The genus *Saccharum* is characterised by both a high ploidy level and aneuploidy and formally comprises six species: *S. spontaneum, S. robustum, S. officinarum, S. barberi, S. sinense and S. edule*. In sugarcane, availability of a large number of varieties invites constant attention in variety identification and assuring genetic purity. Identification of variety and genetic purity assessment based on morphological characters is easy and economical but it may be questionable as they are influenced by factors such as age of the plant material and the environment. Molecular characterization for assigning identification to cultivars can be used in DUS tests, variety registration and dispute settlement. Plant DNA fingerprinting relies on the application of molecular marker techniques to identify cultivars (Hemaprabha *et al.*, 2010). Molecular markers are not influenced by genotype and environment interactions, thus permitting precise identification of genotypes across environments. Among the various markers, sequence-tagged microsatellite sites (STMS) are considered the most efficient in many crops (Kohli *et al*., 2004 and Vir *et al*., 2009). STMS primers are designed from highly conserved flanking regions of simple sequence repeats (microsatellites) which are abundant in plant genomes. Due to its high specificity, STMS markers can distinguish even closely related germplasm lines (Kohli *et al*., 2004). With this point

of view, In this study, fingerprinting was carried out in ten elite sugarcane genotypes commercial value using a set of five sugarcane specific SSR markers.

MATERIAL AND METHODS

Plant Materials

Ten varieties of sugarcane (TNAU Sugarcane Si. 6, TNAU Sugarcane Si. 7, TNAU Sugarcane Si. 8, Co 86032, Co 99004, Co 99006, Co 94008, Co 94012, Co 2001-13 and Co 2001-15) collected from Sugarcane Research Station, Sirugamani and Sugarcane Breeding Institute, Coimbatore were used in this study.

Plant DNA Extraction

DNA was extracted from the young leaves of ten sugarcane varieties raised in the field, which were ground into fine powder in the presence of liquid nitrogen. Genomic DNA was extracted by the CTAB method (Hoisington *et al*., 1994) with minor modification for sugarcane.

PCR Amplification

A total of 5 SSR primer pairs was used in the present study (Table 1). PCR for Simple Sequence Repeat (SSR) analysis performed in a 15 µl reaction volume containing 3.0 µl template DNA, 2 µl PCR buffer, 1 µl ofboth forward and reverse primers, 0.5 µl dNTPs, 0.2 µl Taq- polymerase and 7.3 µl Sterile water. The reactions subjected to the following profile. Initial denaturation at 94˚C for 10 minutes, followed by 30 cycles each of which consisted of 30 Sec denaturation at 94˚C, 45 Sec annealing at 50-56˚C, 45 Sec extension at 72˚C with a final extension at 72˚C for 7 mins. Amplification products were mixed with 3 µl 6X loading dye. PCR products were loaded in each well of 0.8% agarose gel made with 1.0X TAE (Tris Acetate EDTA) buffer and 6µl of 5% ethidium bromide and electrophoresed at 100 volts for 1.5 hours and bands were visualized and documented in gel documentation system. The molecular weight of amplified fragments was estimated with the help of 100 bp plus (MBI, Fermentas) DNA ladder.

Table 1: List of Primer Pairs Used for SSR Analysis

S.No	Marker	Forward Primer	Reverse Primer	Annealing Temp. °C
1	NKSCSSR3 (AFO62734)	CGTGTTCCTCTTCAACAACG	GCGACCGGATTATGATGATT	58
2	NKSCSSR42 (SHY401320)	ACCGATTGTTCAGTGGGAAG	AACCTAGCAATTTACAAGAGAATTAGA	57
3	UGSM32	AGTGAAAGGAGCCAGAAAG	CATTGTTATGCGACTTGTGTT	51
4	UGSM36	GTCATCTCCATCGCCTCCC	CGAAGTTTGGGTCGTTGA	49
5	UGSM49	AGGTTGGCTTGGTGTCTT	TCACTCACAATCACTGGCTAC	49

Data Analysis

The clear and unambiguous bands of SSR were scored. The binary data score was used to construct a dendrogram. The genetic associations between genotypes evaluated by calculating the Jaccard's similarity coefficient for pairwise comparisons based on the proportions of shared bands produced by the primers. Similarity matrix was generated using SimQual programme NTSYS-PC software version 2.02 (Rohlf, 1998). The similarity coefficients were used for cluster analysis and dendrogram was constructed by Unweighted Pair-Group Method with Arithmetic Average (UPGMA).

RESULTS AND DISCUSSIONS

A total of five SSR primer pairs was used to screen ten sugarcane genotypes, out of which one SSR (UGSM36) primer was found to be dimorphic and remaining four were polymorphic. The polymorphism rate estimated to be 36 % to

79% and the Polymorphism Information Content (PIC) value ranged from 0.36 to 0.79. Among the 10 sugarcane genotypes 27 out of 29 bands were polymorphic used among 5 SSR primers. The remaining 2 bands from the 10 genotypes were monomorphic. Out of five primers UGSM32 and NKSCSSR3 only produced monomorphic bands at 250bp and 190 bp respectively (Fig. 1 and Fig. 2). Maximum number of bands were produced by primer UGSM49 and minimum by primer NKSCSSR42. In the present study, the 10 sugarcane genotypes appeared to show difference/variability with the 5 primers used. Therefore, it concluded from the present results that these SSR primers could be used for identification of varieties and assessment of genetic purity of sugarcane.

The dendrogram created by the results of five SSR primers revealed that, Co 2001-15 alone formed a separate cluster at 50% similarity. The similarity coefficient was ranged from 50% to 83%. The three Sirugamani genotypes TNAU Sugarcane Si. 6, TNAU Sugarcane Si. 7 and TNAU Sugarcane Si. 8 converged at 70% similarity and formed a separate cluster. All Coimbatore cultivars except Co 2001-15, converged at 72% similarity and formed another cluster (Fig 3). The pairwise similarity indices showed less similarity was observed between Sirugamani and Coimbatore cultivars and it ranged from 31% to 76%. Among the Sirugamani genotypes and Coimbatore genotypes similarity level ranged from 72 to 79% and 52 to 83% respectively.

Use of microsatellite markers in sugarcane has indicated the power of marker system in characterizing and identifying cane varieties in several countries (Piperidis, 2003). DNA fingerprinting in sugarcane is less complicated in comparison with seed propagated crop as the traits are fixed by vegetative propagation through stem cuttings, thereby facilitating uniformity and stability of the fingerprints once generated (Hemaprabha *et al.*, 2010). Sripunitha (2012) used ten microsatellite markers for identification of fifteen rice varieties. The SSR primer RM320 had the highest efficacy for DNA profiling and varietal identification. Perera *et al.* (2012), reported that in sugarcane among the 36 genotypes, related genotypes tended to group together, as well as those from the same origin in the dendrogram created using 15 SSR primers. In the present study, fingerprinting was carried out in ten elite sugarcane genotypes commercial value using a set of five sugarcane specific SSR markers. Among the five SSRs used, UGSM49 clearly separated the genotypes location wise. Sirugamani genotypes could be separated from Coimbatore cultivars by using this primer. This concluded that the sugarcane genotypes have the similar genetic makeup based on the location.

CONCLUSIONS

Based on this study we concluded that SSR markers are the effective tool for varietal identification and genetic diversity analysis of sugarcane. Among the five SSR primers used, UGSM49 was the unique primer for Co 99006 and NKSCSSR42 was the unique primer for Co 2001-13.

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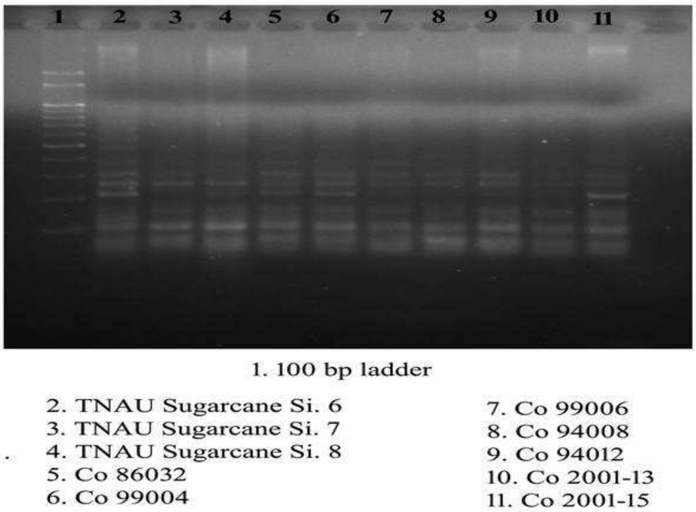

Figure 1: Molecular Characterization of Sugarcane Varieties based on UGSM32 SSR Primer

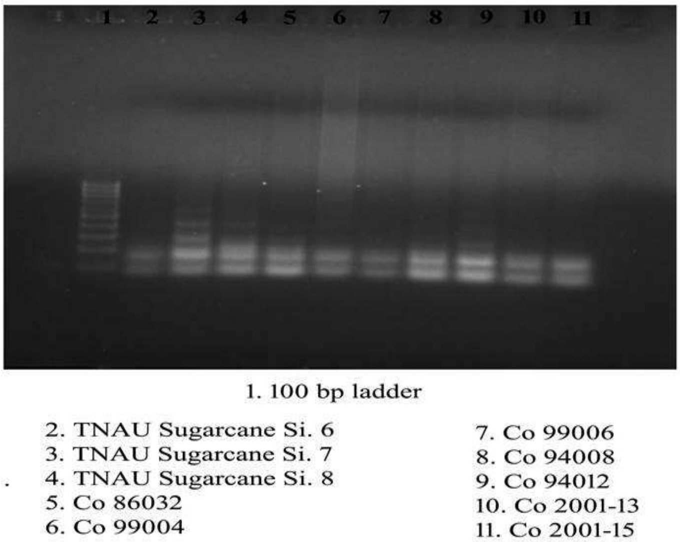

Figure 2: Molecular Characterization of Sugarcane Varieties based on NKSCSSR3 SSR Primer

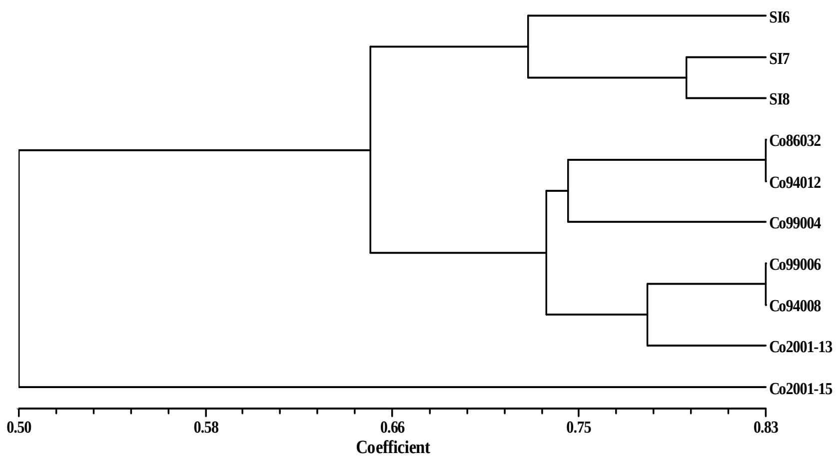

Figure 3: Dendrogram Depicting the Classification of Ten Sugarcane Genotypes Constructed Through UPGMA Method and Based on Five SSR Markers

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